



## New records of *Amanita citrinoannulata* and *A. pakistanica* (Amanitaceae) from India

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### Abstract

*Amanita citrinoannulata* and *A. pakistanica* are reported here as new records for India. Detailed morphological descriptions supported by line drawings, color photo plates and a phylogram based on nrLSU are provided for these two species.

**Keywords** – first report – macrofungi – north western Himalaya – nrLSU – taxonomy

### Introduction

Mushrooms in basidiomycete family Amanitaceae E.-J. Gilbert are generally characterized by agaricoid or secotioid fleshy basidiomes with free to narrowly adnate lamellae, longitudinally acrophysalidic stipe tissue, and bilateral, divergent lamellar trama (Tulloss et al. 2016, Tulloss & Yang 2020). This family comprises five genera i.e., *Amanita* Pers., *Catatrama* Franco-Mol, *Limacella* Earle, *Myxoderma* Kühner and *Limacellopsis* Zhu L. Yang et al. (Cui et al. 2018). The genus *Amanita* is the most dominant in the family Amanitaceae. Generally, *Amanita* is mainly characterized by the presence of universal and partial veils, white to very palid spore print, lamellae edge sterile, and schizohymenial development in its agaric and secotioid species (Bas 1969, Thongbai et al. 2016, Tulloss et al. 2016, Bhatt et al. 2017, Tibpromma et al. 2017). In India, 67 species of *Amanita* are known to date (Bhatt et al. 2017, Tibpromma et al. 2017, Das et al. 2017, Hosen et al. 2018, Mehmood et al. 2018a, b, c, d, 2019, Phookamsak et al. 2019, Semwal et al. 2020, Kumar et al. 2021).

During the course of macrofungal exploration through North-Western Himalaya, a large number of *Amanita* specimens were collected. Macro- and micromorphological examination of our collections revealed two species to be new records for the Indian mycobiota. The nrLSU sequences justified these species to be *Amanita citrinoannulata* Yang Y. Cui, Qing Cai & Zhu L. Yang and *A. pakistanica* Tulloss, S.H. Iqbal & Khalid.

### Materials & Methods

#### Morphological studies

Macromorphological characteristics were documented in the forest or base camp from the fresh and dissected young to mature basidiomata. The photography was accomplished using a digital camera (Sony Cyber-shot W730 and Cannon Power Shot SX 50). Methuen Handbook of

Colour was followed for colour codes and terms (Kornerup & Wanscher 1978). Samples were dried with a field drier. Herbarium codes follow Index Herbariorum (Thiers 2020).

Micromorphological characteristics were observed with the help of a compound microscope (Olympus CH20i) from the dry materials mounted in a mixture of 5% KOH, 1% Phloxin, and 1% Congo red. Biometric variables are after Tulloss & Lindgren (2005) and Tulloss & Rodriguez-Caycedo (2011). Drawings of microscopic elements were made with the Camera lucida at 2000 × magnifications. Microphotography was done with the respective dedicated cameras attached to the compound microscopes: Olympus CH20i and Olympus CX21i LED.

### DNA extraction, PCR and sequencing

Genomic DNA was extracted from dry basidiomes following the modified CTAB method of Doyle & Doyle (1987). PCR was performed to amplify partial sequence of nrLSU using universal primer pairs LR0R (GTACCCGCTGAACTTAAGC) and LR5 (ATCCTGAGGGAACTTC) (Vilgalys & Hester 1990). PCR amplification was conducted on a thermal cycler (Eppendorf, Germany) programmed for 3 min at 94°C, followed by 35 cycles of 30 sec at 94°C, 1 min at 55°C, 1 min at 72°C and a final stage of 8 min at 72°C. The PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN, Germany). Both strands of the PCR fragments were sequenced on the 3730xl DNA Analyzer (Applied Biosystems, USA) using the amplifying primers.

### Phylogenetic analyses

Multiple sequence alignment was performed using MAFFT v.7 (Kato et al. 2005) with minimal editing in BioEdit v.7.2.5 (Hall 1999). Phylogenetic analysis was undertaken based on maximum likelihood (ML) in MEGA 6.0. (Tamura et al. 2013). Default settings were used for all parameters in the ML analysis and statistical support values were obtained using nonparametric bootstrapping with 500 replicates. *Amanita singeri* Bas and *A. vittadinii* (Moretti) Vitt. were selected as an outgroup for the molecular phylogenetic analysis.

## Results

### Phylogenetic analyses

The nrLSU dataset consisted of 62 sequences including our two sequences derived from the studied species. In the nrLSU phylogenetic tree our Indian specimen (RET-716-5) *Amanita citrinoannulata* (GenBank accession number MW063469) sequence clustered with *A. citrinoannulata* (GenBank accession number NG\_064565) sequences from China with strong support (96% BS). The sequence of the specimen (RET 411-7 (GenBank accession number MG991814) nest amongst *Amanita pakistanica* (voucher RET 317-6) with strong support (99% BS). Also, the closest Blast hit for the ITS sequence of our specimen from India AK-10 (accession number MW073431) is the sequence KX061525 (*Amanita pakistanica* voucher RET 352-1), with 100% identity and 100% query cover.

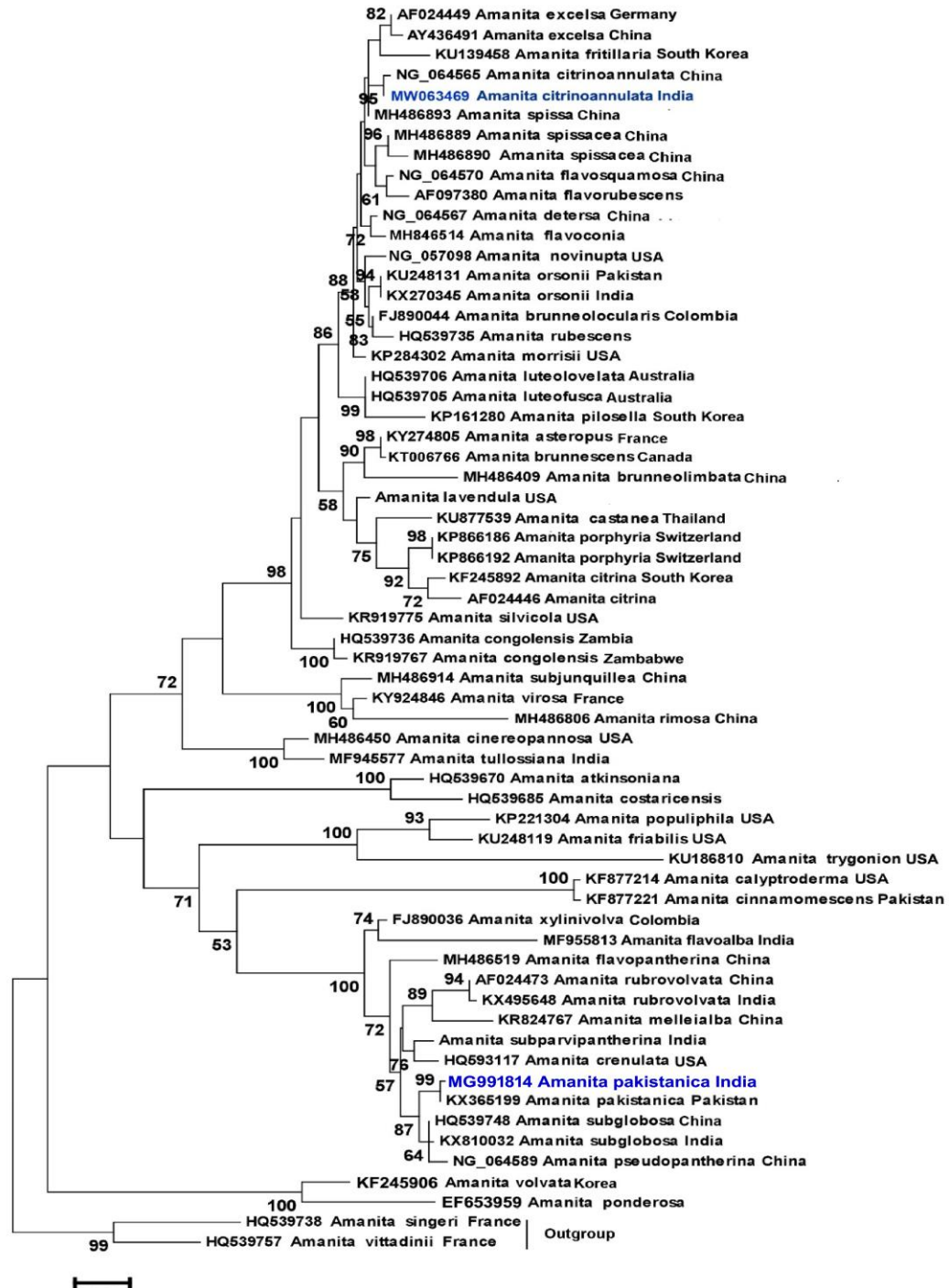
### Taxonomy

*Amanita citrinoannulata* Yang Y. Cui, Qing Cai & Zhu L. Yang, Fungal Diversity 91: 182 (2018)  
Figs 2, 3

Facesoffungi number: FoF 0101

*Basidiomata* small to medium sized. *Pileus* 40–78 mm wide, initially hemispherical then convex to plano-convex and finally plane, yellowish brown (5D5) to olive brown (4E5-7) to olive (3F5-7) at centre, yellowish grey (4B2) to greyish yellow (4B2) toward margin, with surface having dry shiny appearance; *context* 5–7 mm thick, thinning slowly toward margin, white, turning reddish when cut or bruised; *margin* non-striate, non-appendiculate. *Universal veil on pileus* as felted to granular patches, olive (4F6) to olive brown, uneven distributed. *Lamellae* free, crowded (10–12 lamellae/10 mm at margin) cream, 4–6 mm broad, turning reddish when cut or bruised. *Lamellulae*

attenuate, of several lengths, plentiful. *Stipe* 55–125 × 21–24 mm, yellowish white, solid, cylindrical or narrowing upward, fibrils arranged in zebroid pattern above partial veil, powdery fibrils below. *Bulb* 17–30 × 21–31 mm, ovoid, covered by loose powdery-floccose, yellowish brown universal veil remnants. *Partial veil* apical to superior, membranous, inner side reddish tinge, light lemon-yellow outer side, soft yellowish grey cottony patches on the underside. *Spore print* white.



**Fig. 1** – Maximum likelihood phylogenetic tree of *Amanita* sect. *Amanita* nrLSU sequences, showing the positions of *A. pakistanica* and *A. citrinoannulata*. New records are in blue font in the tree. Bootstrap support values equal to or higher than 50% are mentioned above branches.

*Basidiospores* (7–)8.0–11(–11.5) × (6.0–)7.0–8.0(–8.5) μm, L = 8.5–10.5 μm; L' = 9.3 μm; W = 6.5–7.5 μm; W' = 7.10 μm; Q = (1.14–)1.23–1.36(–1.42), Q = 1.25–1.35; Q' = 1.29), broadly

ellipsoid, slightly thin-walled, hyaline, amyloid, *contents* monogutulate, with apiculus sublateral. *Basidia* (30–)35–45(–56) × (8.0–)9.5–11.5(–12) μm, thin-walled, 2-4 spored, sterigmata up to 4 × 2 μm; basal clamps absent. *Subhymenium* *w<sub>st-near</sub>* = 26–56 μm thick, *w<sub>st-far</sub>* = 48–68 μm, basidia arise from ovoid to irregular shape cells 6–12 × 5–10 μm wide. *Hymenophoral trama* bilateral, divergent; *w<sub>cs</sub>* = 40–70 μm, well rehydrated, filamentous undifferentiated hyphae 4–16 μm wide. *Pileipellis* slightly gelatinizing, filamentous, undifferentiated hyphae 2–7 μm wide, thin-walled, filamentous undifferentiated hyaline, mainly radially to compactly arranged, vascular hyphae 6–8 μm wide. *Pileus context* filamentous, undifferentiated hyphae 4–12 μm wide, thin-walled, hyaline; inflated cells up to 180 × 50 μm, thin-walled, hyaline, colourless. *Universal veil on the pileus* filamentous, undifferentiated hyphae 4–12 μm, thin-walled, hyaline, branched, inflated cells; globose to subglobose 40–106 × 32–92 μm, broadly ellipsoid to cylindrical 55–95 × 24–60 μm, infrequent, thin-walled, hyaline. *Universal veil on the stipe* base similar to pileus surface. *Partial veil* filamentous, undifferentiated hyphae dominant 2–7 μm wide; with a cluster of inflated cells clavate to cylindrical up to 18–28 × 90–160 μm; globose to subglobose 19–24 × 16–34 μm. *Stipe context* acrophysalidic, with acrophysalides up to 30 × 198 μm wide, filamentous, undifferentiated hyphae 3–12 μm wide. *Clamp connections* absent in all tissues.

Habit and Habitat – Solitary to scattered under *Abies pindrow* in mixed coniferous-broad leaves forest in Western Himalaya.

Known distribution – This species was originally described from China (Cui et al. 2018), and is now known also from India.

Specimens examined – INDIA, Uttarakhand, Rudraprayag district, Chopta-Baniyakund, 10 August 2014, *T. Mehmood*, TM-14-345; Bagashwar district, Dhakuri 02 Aug. 2016, *T. Mehmood*, TM-15-1254; Rudraprayag district, Chopta-Baniyakund, 01 Aug. 2017, *T. Mehmood*, TM-16-1495; Chopta-Baniyakund, 09 September 2017, *T. Mehmood*, TM-17-1612; Chopta-Baniyakund, 07 Aug. 2017, *T. Mehmood*, TM-16-1613, Jammu & Kashmir, Kathua district, Bani-Sarthal, 10-07-2019, A. Kumar, AK-19-0010.

Notes – *Amanita citrinoannulata* was described from China and is characterized by brown to gray-brown pileus with an olivaceous tinge, yellow, snakeskin shaped squamules on the stipe surface above the annulus, an ellipsoid, ventricose to subglobose stipe base covered with floccose, yellow volval remnants, a median citrine annulus, broadly ellipsoid to ellipsoid basidiospores (7.0–9.5 × 5.5–7.0 μm), and red, reddish to pinkish discolorations over all parts of the basidioma when it is bruised or injured (Cui et al. 2018). However, collections from India have yellowish brown to olive-brown pileus with yellowish-grey to greyish-yellow margin, stipe surface above partial veil covered with fibrils arranged in a zebroid pattern, an ovoid to subglobose bulb covered with floccose, yellowish-grey warts and basidiospores 8.0–11 × 7.0–8.0 μm (Q = 1.25–1.35; Q' = 1.29). These differences are part of the variability of species.

Morphologically and phylogenetically, the present taxon resembles the European species *A. spissa* (Fr.) P. Kumm. (Fig. 1). However, *A. spissa* differs from *A. citrinoannulata* by its large basidioma, a grey pileus lacking an olivaceous tinge and a white to dirty white universal veil remnants (Contu 2000, Neville & Poumarat 2004, Yang 2015).

Two rubescent taxa of section *Validae*; *Amanita rubescens* var. *rubescens* and *A. flavorubens* (Berk. & Mont.) Sacc. are somewhat close to *A. citrinoannulata* but *Amanita rubescens* var. *rubescens* differs from *A. citrinoannulata* by its brown pileus and ellipsoid to elongate basidiospores (8–10.6 × 5.5–7.0 μm) whereas *A. flavorubens* is separated from *A. citrinoannulata* by its brown to yellow pileus covered with patchy, lemon yellow volval remnants (9.4–10.2 × 6.2–7.0 μm) (Jenkins 1982, 1986, Tulloss et al. 1995).

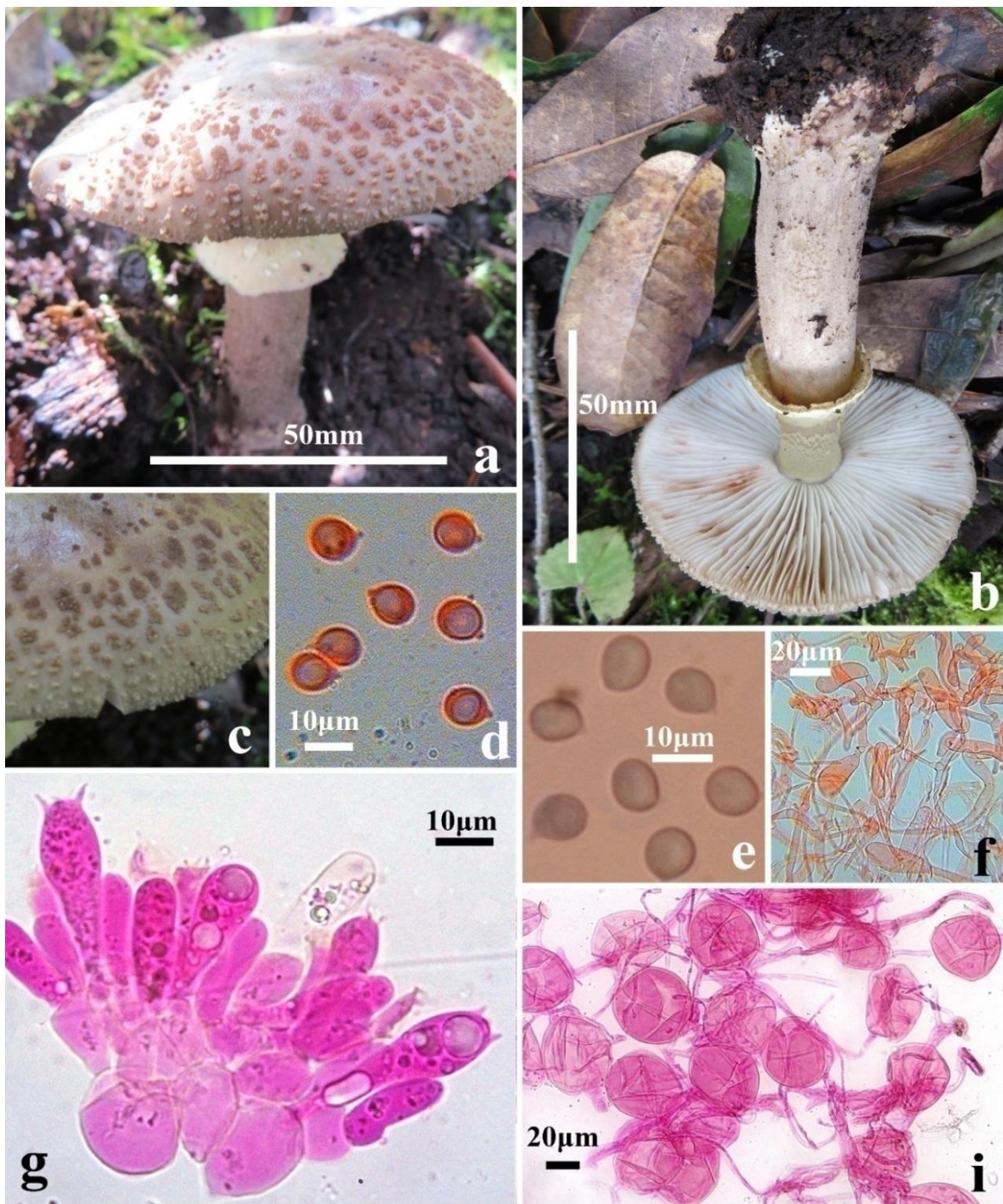
***Amanita pakistanica*** Tulloss, S.H. Iqbal & Khalid, Mycotaxon 77: 458 (2001)

Figs 4, 5

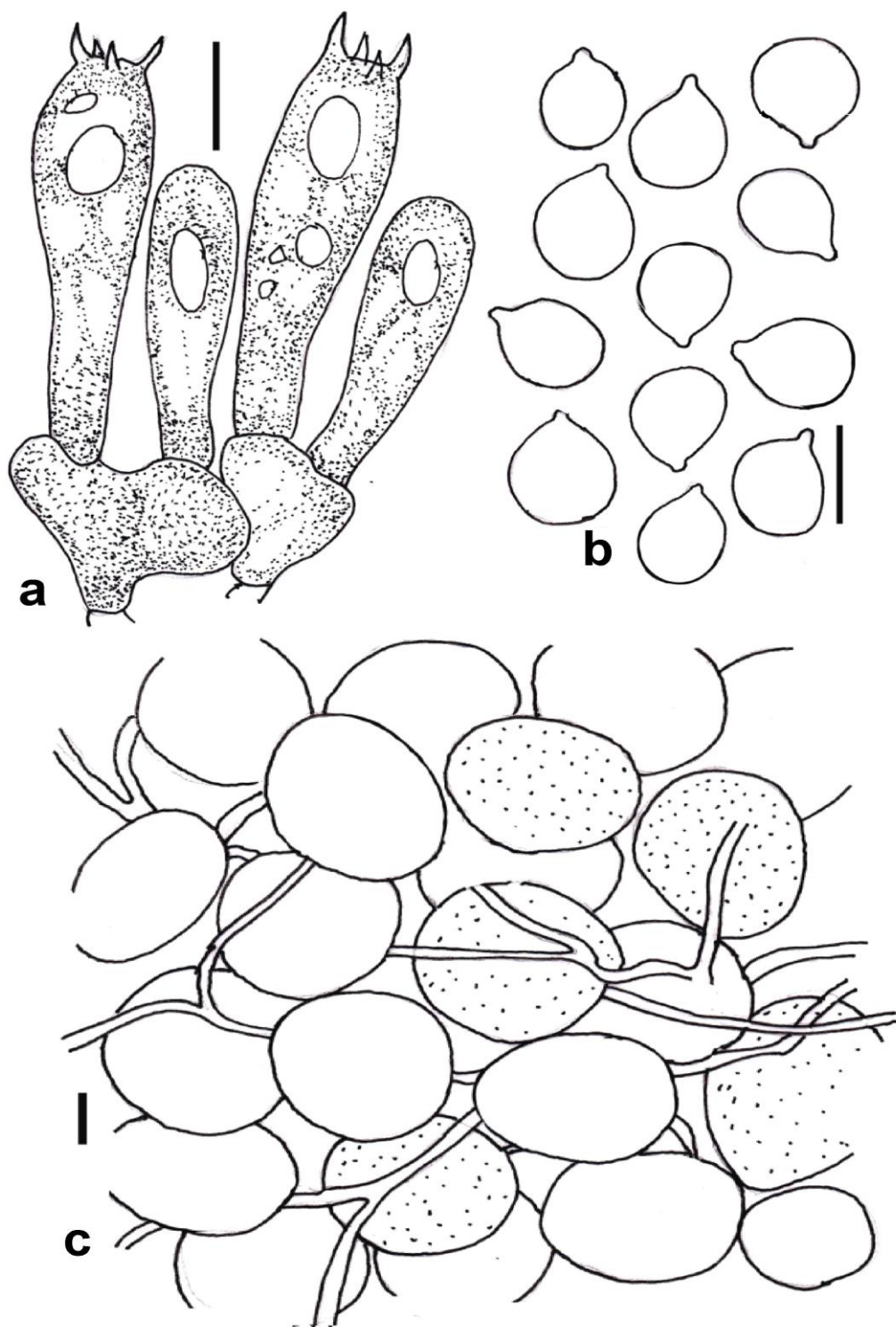
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*Basidiomata* small to medium size. *Pileus* 45–89 mm wide, hemispherical when young, then convex to plane at maturity, shining, sometimes viscid when moist, pale yellow (3A3) light brown (6D5) at centre, pale yellow (1A3) toward margins. *Universal veil on pileus* white (2A1), granular

to sub-conical warts. *Pileus margin* tuberculate striate, striation up to (0.10–0.20 R) non-appendiculate. *Pileus context* 4–6 mm thick at centre, white to off-white. *Lamellae* free, crowded (7–10 lamellae/10mm at margin), white (1A1). *Lamellulae* attenuate, of several lengths, plentiful, few forked. *Stipe* 85–120 × 12–16 mm, tapering upward, white white (1A1) to yellowish white (1A2) unchanging when exposed or bruised, with white fibrils above and below annulus. *Stipe context* white (1A1), stuffed, unchanging when cut or bruised. *Partial veil* superior to median, whitish to yellowish white (4A2), striate above, covered by pale yellow warts. *Basal bulb* 27–38 × 18–25 mm, remnants of universal veil on top of the bulb irregularly arranged, white (1A1) to yellowish white (1A2). *Odour* indistinct. *Taste* not recorded. *Spore print* white.



**Fig. 2** – *Amanita citrinoannulata*. a–b Fresh basidiomata in the field. c Universal veil remnants on pileus. d–e Basidiospores. f Elements of partial veil. g Hymenium and subhymenium. i Elements of universal veil from pileus surface.

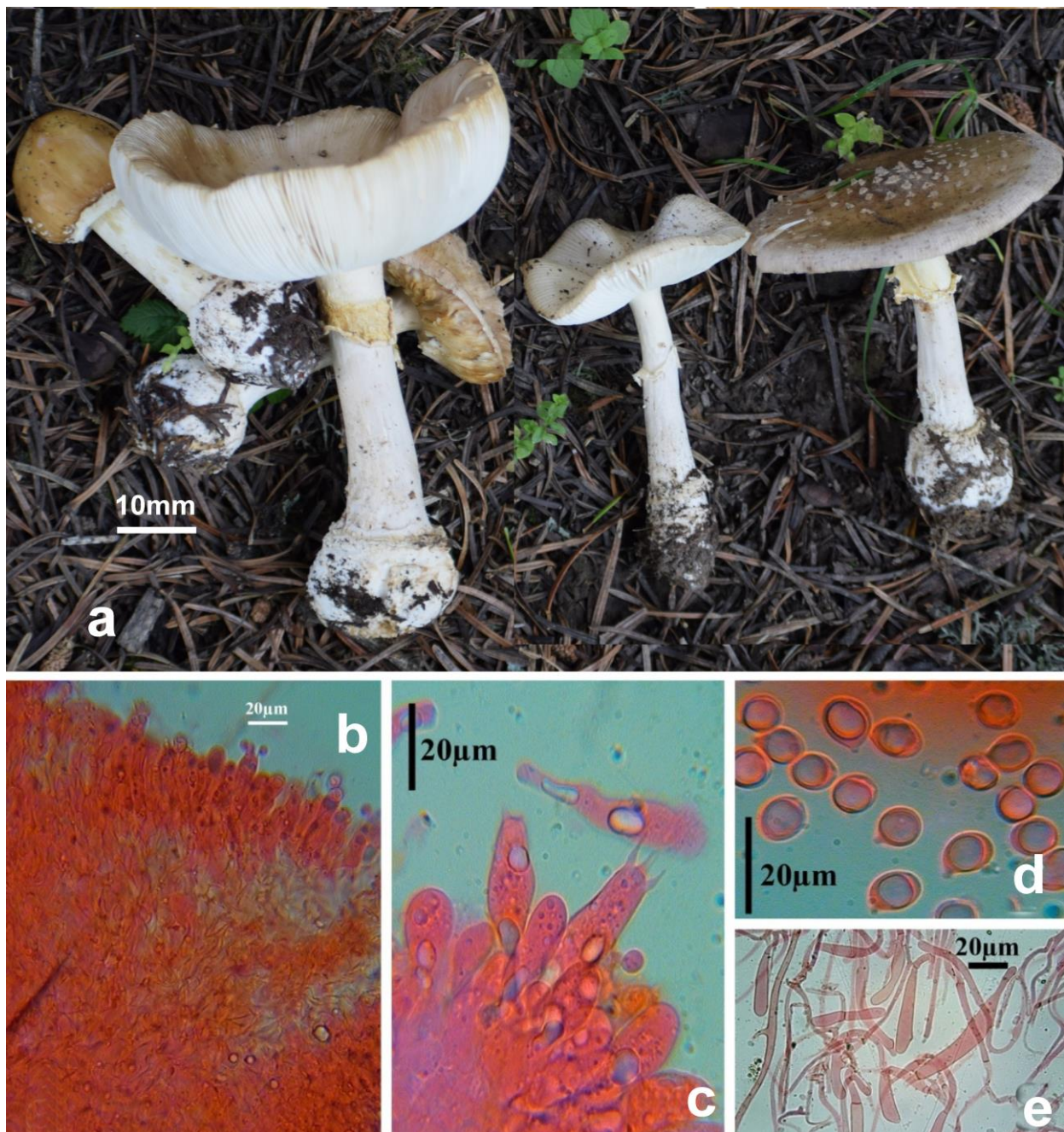


**Fig. 3** – *Amanita citrinoannulata*. a Hymenium and subhymenium. b Basidiospores. c Elements of universal veil from pileus surface. Scale bars: a–c = 10  $\mu$ m.

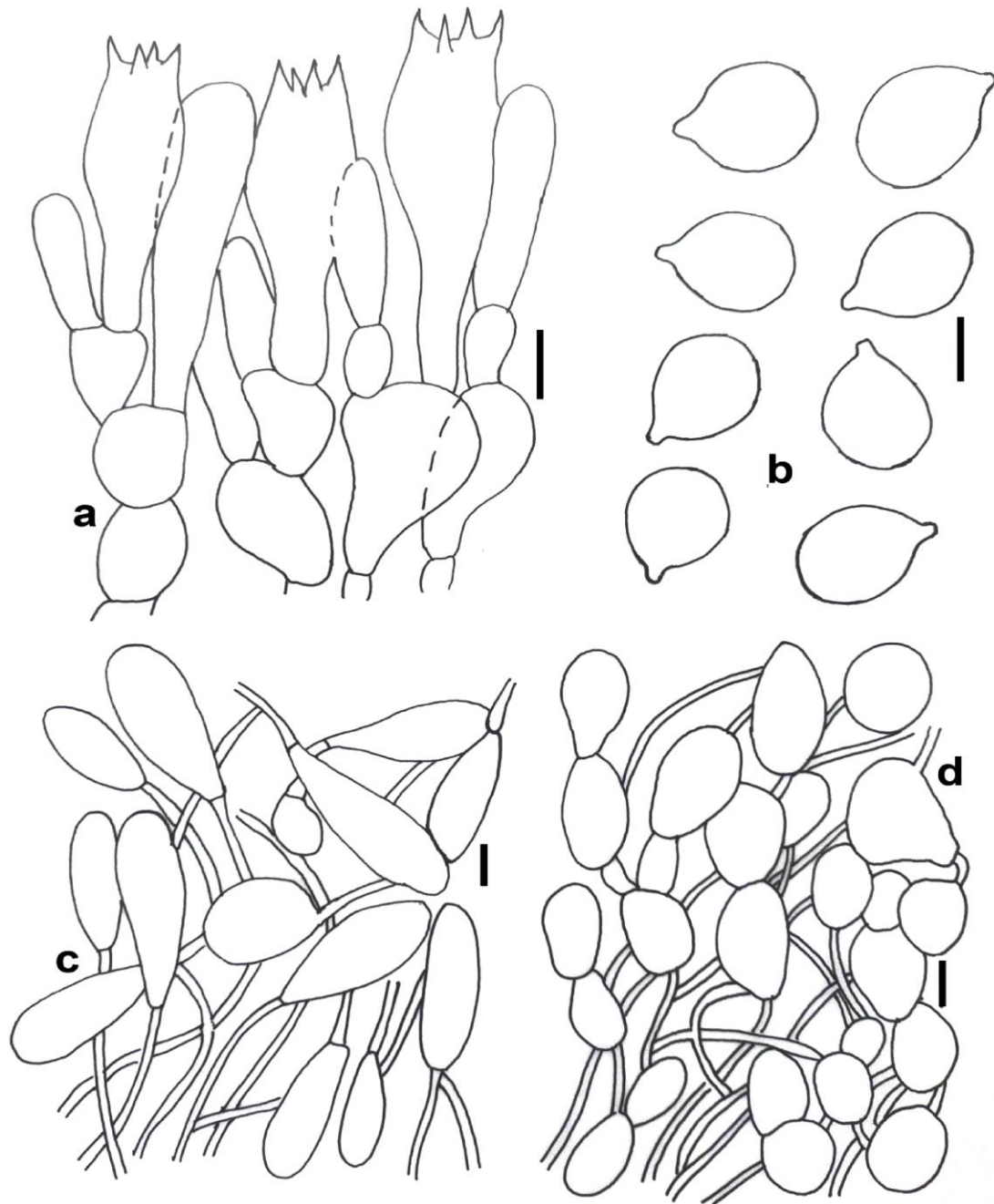
Basidiospores (8.0–)8.5–12(–12.5)  $\times$  (6.0–)7.0–8.5(–9.5)  $\mu$ m, L = 9.5–11.5  $\mu$ m; L' = 10.5  $\mu$ m, W = 7–8  $\mu$ m; W' = 7.6  $\mu$ m; Q = (1.20–)1.26–1.50(–1.55); Q = 1.33–1.50; Q' = 1.40, thin walled, hyaline, smooth, broadly ellipsoid to ellipsoid inamyloid, apiculus sublateral, up to 1.3  $\mu$ m; contents monoguttulate. Basidia (40–) 45–55(–58)  $\times$  (9.0–)10–12(–13)  $\mu$ m, thin-walled, colourless, sterigmata up to 3–4  $\mu$ m long; basal clamp absent at the base of basidia. Subhymenium  $w_{st-near}$  = 15–28  $\mu$ m;  $w_{st-far}$  = 28–40  $\mu$ m. Lamellar edge cells sterile, with inflated clavate up to 22–25  $\times$  9–10  $\mu$ m, colourless. *Hymenophoral trama* bilateral, divergent;  $w_{cs}$  = 40–65  $\mu$ m; inflated cells up to

82 × 30 μm, filamentous, undifferentiated hyphae 5–7 μm wide, thin-walled, hyaline. *Pileipellis* 105–140 μm thick, filamentous, undifferentiated hyphae 3–4 μm wide, radially interwoven. *Pileus context* filamentous, undifferentiated hyphae 5–8 μm wide, septa, thin-walled, hyaline, broadly ellipsoid to ellipsoid cells 130–190 × 20–25 μm, thin-walled, colourless; vascular hyphae not observed. *Universal veil on pileus* filamentous, undifferentiated hyphae 4–5 μm wide, hyaline; inflated cells subglobose to broadly ellipsoid up to 30 × 40 μm, vascular hyphae up to 8 μm wide. *Partial veil* filamentous, undifferentiated hyphae 4–8 μm wide; inflated cells; ellipsoid up to 85 × 18 μm. *Stipe context* longitudinally acrophysalidic; acrophysalides 190–350 × 30–38 μm; filamentous, undifferentiated hyphae 5–10 μm wide, thin-walled, hyaline. *Clamp connections* absent in all tissues.

Habit and Habitat – Solitary to scattered on the ground in a mixed forest of *Abies pindrow*, *Quercus semecarpifolia*, *Cedrus deodara* etc.



**Fig. 4** – *Amanita pakistanica*. a Fresh basidiomata in the field. b Hymenium & subhymenium. c Basidia at different stages of development. d Basidiospores. e Elements of partial veil.



**Fig. 5** – *Amanita pakistanica*. a Hymenium & subhymenium. b Basidiospores. c Elements of partial veil. d Elements of universal veil. Scale bars: a–d = 10  $\mu$ m.

Known distribution – This species was originally described from Pakistan (Tulloss et al. 2001), and is now known from India.

Specimens examined – INDIA, Uttarakhand, Pauri district, Phedkhal, 16 July 2016, *T. Mehmood*, TM-16-1094; Phedkhal, 19 July 2016, *T. Mehmood*, TM-16-1320; 25 August 2016, *T. Mehmood*, TM-16-1360; Phedkhal, 03 September 2016, TM-16-1387; Rudraprayag district, Chopta, 27 September 2016, *T. Mehmood*, TM-16-1412.

Notes – *Amanita pakistanica* belongs to (*A.* subgenus *Amanita*) sect. *Amanita*. It is characterized by its small to medium-sized basidiomata, pale yellow to dull yellow pileus over centre, whitish yellow to pale yellow toward margin, white stipe and universal veil at stipe base in form of white granular warts and broadly ellipsoid to ellipsoid basidiospores. The size of



basidiomata, as well as size and shape of basidiospores of the Indian collections is very similar to the original description from Pakistan (Tulloss et al. 2001).

*Amanita pakistanica* is somewhat similar to *A. parvipantherina*. However, the latter possesses a brownish pileus with shorter marginal striations (Yang et al. 2004, Yang 2005, 2015). *Amanita pakistanica* resembles *A. pseudosychnopyramis* by its small to medium-sized basidiomata but latter can be easily segregated by its conical to pyramid, grey to brownish grey universal veil remnants on the pileus, a basal bulb with a short limbate collar and its subglobose to broadly ellipsoid basidiospores  $8.5\text{--}10 \times 7.5\text{--}8.5 \mu\text{m}$  (Ariyawansa et al. 2015). The morphological characteristics of Indian collection is very similar to the original description. It constitutes a new record for India.

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